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SPONTANEOUS MUTATION AT THE *R* LOCUS IN MAIZE. I. RACE DIFFERENCES IN MUTATION RATE

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THE frequency of spontaneous mutation of *R* differs rather widely in different races or stocks. Such differences may be due either to (1) intrinsic differences in mutability of the *R* alleles in the stocks compared, or (2) differences between the stocks in characteristics which affect *R* mutation frequency. This report is concerned with the extent and nature of these differences.

MATERIALS AND NOTATION

Among the alleles designated R^r (colored seed, colored plant), there are several types with distinguishable phenotypic effects. A study of 22 R^r's extracted from more or less isolated races indicated that few if any of the alleles compared were identical (Stadler and Fogel, 1943, 1945). The 22 specimens of this sample fell into four major groups, distinctly differing in intensity and distribution of plant pigmentation. Within these groups minor quantitative differences were found, differences which may be demonstrated by objective tests in comparisons

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in which environmental and genetic modification is minimized, but which are much too small to permit identification of the allele from the appearance of a single plant. By conventional standards, the types represented by the four major groups may be designated as different alleles of *R*. There are in addition other phenotypically distinguishable alleles of *R*, with colored seed and colored plant effects, which were not included in this sample. Revision of the symbol-system for the *R* locus will be considered in another paper.

For the purposes of this study of mutation rates, none of the alleles of separate origin may be assumed to be identical, regardless of phenotypic similarity. In this report the designation, R^r, is used for all alleles showing seed and plant color effects, with the addition of a distinguishing name for each allele of separate origin. Similarly the designation, R^g, is used for alleles showing seed color without plant color effect, with the addition of a distinguishing name for alleles of separate origin.

Among the R^r alleles included in this study, there are representatives of three of the four major phenotypic groups mentioned above. The group most closely corresponding in phenotype to description of R^r in the literature, here designated group *A*, includes R^r : Boone, R^r : Cornell, R^r : Quapaw, R^r : Ponca, R^r : Black. These are characterized by fairly strong pigmentation of certain tissues in the seedling stage, and in the mature plant from the flowering stage onward. Group *B*, represented by R^r : Carrion and R^r : Young, is similar to group *A*, except for stronger pigmentation of certain tissues, notably in the pericarp, silks and auricles, and in general slightly weaker pigmentation elsewhere. Group *D*, represented by R^r : Arapaho, R^r : Chickasaw, R^r : Pampa, R^r : Winnebago, R^r : Catspaw and R^r : Deaurle, has seedling pigmentation not much less intense than that of groups *A* and *B* in some of its members, though ranging to a distinctly lower level in others. Seedlings of group *D* lack leaf-tip color, which is invariably present in seedlings of groups

A and *B*. At the flowering stage, plants of group *D* are almost devoid of anthocyanin, and are sometimes indistinguishable from R^g plants. The members of this group of R^r alleles show pronounced intensification of mature plant color effect in the presence of certain specific modifiers which are without effect upon the action of R^r alleles of groups *A* and *B*.

The stocks from which the R^r alleles were extracted represent a group of races probably unrelated in recent descent. The collection of stocks from American Indian reservations was secured through the kindness of J. H. Kempton, then of the U. S. Department of Agriculture, who had collected them some years earlier and had maintained them through 4–6 generations of selfing and sibbing. This group was supplemented by three additional R^r stocks, (1) "Black," an inbred line of Black Beauty popcorn, (2) "Cornell," a stock originally secured in 1926 as a genetic tester stock but subsequently maintained without outcrossing (chiefly by self-pollination) for ten or more generations, and (3) "Boone," a genetic tester stock in current use.

Mutation Rate in Different Stocks

Determination of the mutation rate in the pure stock was not feasible for most of the R^r alleles. The chief reason for this was the occurrence, in many of the stocks, of variable aleurone coloration occasionally ranging to light or nearly colorless aleurone. In the homozygous stocks colorless seeds did not occur, and seeds of distinctly light coloration were rare, but in the heterozygous *R r* seeds produced for determination of mutation rates the weakening of color expression was more conspicuous. In many cases a large proportion of the ears were found to be unsuitable for examination for seed-color mutation, and among those considered suitable most of the seeds tested as suspected mutants were disproved by the test. The weakened and variable aleurone color expression appears to be due to a factor linked with *R*,

with sufficiently frequent crossing over to permit the extraction of strains suitable for mutation study. It is feasible, therefore, to determine the mutational behavior of the R^r alleles of this group, but not to determine mutation rates in the original stocks. Another difficulty in determining mutation rate in the pure stocks was the very low productivity of many of these stocks.

Data on mutation frequency in the pure stocks for all stocks in which more than 5000 gametes were tested are shown in Table I.

TABLE I. FREQUENCY OF SEED-COLOR MUTATION RATE OF *R* IN VARIOUS STOCKS

Stock	Allele	Mutation Frequency	Mutation Rate	Limits of Expectation ($P = 0.05$)
			(× 10^{-4})	
Boone	R^r (group *A*)	47/110,060	4.3	5.1– 3.7
Cornell	R^r (group *A*)	79/ 43,416	18.2	20.7–16.4
Quapaw	R^r (group *A*)	23/ 40,845	5.6	7.3– 4.7
Black	R^r (group *A*)	6/ 11,930	5.0	10.9– 1.8
Young	R^r (group *B*)	0/ 19,257	0	1.9– 0
Arapaho	R^r (group *D*)	0/ 9,730	0	3.8– 0
McBaine	R^g	0/ 16,617	0	2.2– 0

It is noteworthy that no seed-color mutations were observed except in stocks carrying R^r alleles of group *A*. Among these the Cornell stock yielded mutations at a rate several times as high as the rates found for the other stocks tested.

A word of explanation should be added regarding the method used in this table, and in the succeeding tables, to indicate the sampling errors involved in the mutation rates given. Stevens (1942, 1945) has pointed out the gross error which may be involved in estimates of the sampling error for mutation rates based upon small numbers of mutants observed, when conventional criteria of significance are applied. He has provided a table to facilitate the accurate determination of the limits of expectation, based upon the binomial distribution. The values given in the column headed "Limits of Expecta-

tion ($P = 0.05$)'' represent the range in mutation rates not contradicted by the observation, at this level of significance. The abbreviation ''L. E.,'' used for this expression in subsequent tables and in the text, refers to limits at the $P = 0.05$ level unless otherwise stated.

Relative Frequency of Plant-Color Mutation and Seed-Color Mutation in Different R^r Alleles

The frequency of R^r mutation affecting plant color expression is measured in the same way as that affecting seed color expression, but since this requires the examination of a seedling rather than the examination of a seed for each gamete tested, it is not feasible to determine mutation rates in such large populations.

In the course of various experiments with R^r: Cornell, large populations of seedlings have been examined and a considerable number of mutations to R^g identified. Smaller populations have been examined for plant-color mutations of other R^r alleles, and mutations to R^g have been identified in several of these.

Six mutations of R^r: Columbia to R^g have previously been reported (Stadler, 1946). The allele R^r: Columbia, which was not included in the gene variability study, is similar in phenotypic expression to the R^r alleles of group *A*.

The data summarized in Table II include all additional data available for plant-color mutation rate in R^r alleles grown in homozygous cultures. In many cases these were homozygotes extracted from crosses of the R^r stock concerned with some distinguishable R^g or r^r type. Since the seed-color mutation rate in the extracted stock may differ materially from that found in the original stock, the table includes also data on seed-color mutation rates in the same cultures, for comparison.

In spite of the rather small numbers tested for plant-color mutation, the data suggest some difference in plant-color and seed-color mutation behavior of the alleles compared. The alleles of group *B*, R^r : Carrion and R^r :

Young, which have failed to give seed-color mutations in populations of substantial size, gave 6 plant-color mutations, the highest plant-color mutation frequency observed being that of R^r: Young.

TABLE II. FREQUENCY OF PLANT-COLOR MUTATION AND SEED-COLOR MUTATION OF VARIOUS R^r ALLELES

Allele	Plant-Color Mutation			Seed-Color Mutation		
	Mutation Frequency	Mutation Rate ($\times 10^{-4}$)	(L. E.)	Mutation Frequency	Mutation Rate ($\times 10^{-4}$)	(L. E.)
R^r: Boone	0/ 4,928	0	7.5–0	14/ 32,085	4.4	7.3–2.4
R^r: Cornell	30/71,281	4.2	5.3–3.9	94/109,904	8.6	9.6–7.8
R^r: Quapaw	0/ 3,150	0	11.7–0	12/ 30,084	4.0	7.0–2.1
R^r: Ponca	0/ 3,758	0	9.8–0	7/ 5,339	13.1	27.0–5.3
R^r: Black	1/ 3,342	3.0	16.7–0.1	4/ 5,936	6.7	17.3–1.8
R^r: Carrion	2/10,924	1.8	6.6–0.2	0/ 25,911	0	1.4–0
R^r: Young	4/ 5,769	6.9	17.8–1.9	0/ 8,432	0	4.4–0

The plant-color mutations, like those previously reported in R^r: Columbia, were identified by seedling examination for leaf-tip color. R^r alleles of group *D* lack seedling leaf-tip color, and any mutation to this level among the group *A* and *B* alleles tested would have been detected. As in the previous study with R^r: Columbia, all of the plant-color mutations found were mutations to R^g, wholly devoid of anthocyanin.

The R^g mutants of R^r: Carrion and R^r: Young were all checked for pericarp color effect in the presence of *Pl*, since R^r : Carrion and R^r : Young are distinguished from the R^r allele of group *A* chiefly by their stronger effect upon pericarp color. All six R^g mutants of this group, like those of group *A*, showed complete absence of anthocyanin in the pericarp.

DIRECT COMPARISON OF MUTABILITY OF *R* ALLELES IN HETEROZYGOTES

When the mutants of one *R* allele can be regularly distinguished from those of another, it is possible to compare the mutability of the two alleles directly under

strictly comparable conditions in the heterozygote. The fact that, for certain R^r alleles at least, mutations of seed-color and plant-color effect occur independently (Stadler, 1946), makes such comparisons possible in certain cases. In the heterozygote R^r/R^g the two R alleles compared are present in equal numbers, and the environment and the modifier genotype are the same for both. Assuming that seed-color mutations of R^g would have no effect upon plant-color expressions, the seed-color mutants produced by the heterozygote may be classified as to origin, the r^r mutants being ascribed to mutation of the R^r allele, and the r^g mutants ascribed to mutation of the R^g allele with the exception of the small proportion expected from mutation of the R^r allele. The assumptions underlying this classification are somewhat questionable, for there may be some interaction between the two R alleles present, such as to make their mutational behavior different from that observed in homozygotes.

An F_1 produced by crossing the Cornell stock with the McBaine stock yielded 19 colorless seeds among 83,885 seeds examined. Two of the colorless seeds failed to germinate, and the remainder were identified as 15 mutations to r^r and two mutations to r^g. If the relative frequency of mutation of R^r: Cornell to r^g is as low in the heterozygote $R^r\ R^g$ as in the homozygote $R^r\ R^r$, it is probable that both r^g mutations were derived from the R^g gene. Since the total mutation rate for the R^r: Cornell gametes in the F_1 is much lower than in the parental R^r stock, this assumption is rather dubious, for with pronounced reduction of the frequency of mutation to r^r and with no reduction in the frequency of the mutation to r^g, the total population of R^r: Cornell gametes tested might have yielded one or two mutations to r^g.

Regardless of the origin of the two r^g mutants, it is evident that the R^r gametes in the heterozygote mutated at a rate substantially higher than that of the R^g gametes.

The McBaine stock carries the recessive gene *g* (golden

plant color) which is linked with *R* with about 14% crossing over. At the time, no r^g *g* stock was available for use as a pollen stock in determining mutation frequency or as a tester in confirming the mutation. The presence of *g* was not determined for the mutations in general, but after the confirmation of the two r^g muta-

TABLE III. FREQUENCY OF SEED-COLOR MUTATION OF *R* IN R^r/R^g HETEROZYGOTES

Genetype	Mutation Frequency	Mutants		
		r^r	r^g	Undet.
R^r: Cornell/R^g: McBaine	9/79,858	6	2	1
R^r: Ponca/R^g: McBaine	4/16,765	4		
R^r: Black/R^g: McBaine	2/10,420	2		
R^r: Young/R^g: McBaine	0/ 8,834			
R^r: Carrion/R^g: McBaine	0/10,160			

tions they were tested for *g*, and in each of the two cases it was found that the chromosome carrying the mutant gene in the mutating R^r/R^g *g* plants was r^g *g*.

While this trial indicates that the difference in mutation frequency between R^r: Cornell and R^g: McBaine is due in part to the intrinsically higher mutability of R^r: Cornell, it also indicates that the Cornell stock owes its high mutability in part to the presence of modifier genes favorable to mutation. Of the 83,885 gametes tested for mutation frequency in the F_1 culture, about half were R^r: Cornell gametes. A population of 42,000 tested gametes in the original Cornell stock would have yielded about 75 seed-color mutations, while in the F_1, with half of the residual genotype derived from another stock, the yield of mutations from the R^r gametes was certainly not over 19.

A further trial was made with several of the R^r alleles in F_1's with R^g: McBaine, in which it was possible to determine the *g* constitution of the chromosome bearing the mutant *r* for all of the mutants except two which failed to produce an ear for a progeny test. The relative frequency of r^r and r^g mutants is shown in Table III.

Again mutations to r^r are distinctly more frequent than mutations to r^g. The data for the individual R^r alleles, in comparison to R^g: McBaine, are not statistically significant, but are in agreement with the data from R^r: Cornell in the previous trial in indicating the relatively low mutability of the R^g allele. It is interesting to note that the mutation rates of R^r: Ponca and R^r: Black do not show the sharp reduction following crossing with the McBaine stock, which was found in both crosses in the case of R^r: Cornell.

The tests for g constitution further classify the mutants in relation to the R^r G and R^g g chromosomes present in the parent plants. Of the 10 r^r mutants tested, 9 were r^r G and 1 r^r g; of the 2 r^g mutants, one was r^g G and one r^g g. In addition, as previously mentioned, the 2 r^g mutants of the earlier R^r: Cornell/R^g: Mc Baine culture were both r^g g. Thus among 14 mutants tested, 2 occurred in chromosomes in which crossing over occurred between R and g, and 12 in chromosomes without crossing over in this interval. This is as expected if mutation of R is not related to crossing over. It supports the assumption that the r^g mutants are chiefly the result of mutation of the R^g allele, and that the r^r mutants are the result of mutation of the R^r allele.

The same method may be used for direct comparison of the mutability of R^r alleles of group D with that of alleles of group A. The seedling phenotype of the group D alleles is readily distinguished from that of group A alleles by the absence of leaf-tip color. Four alleles of this group were crossed with R^r: Cornell, and tests of seed-color mutation were made in the usual manner.

In most of the alleles of group D, including the four used in this experiment, attempts to determine the frequency of seed-color mutation had been hampered by the tendency toward variable seed-color expression previously mentioned page 291). This tendency is apparent also in F_1 progenies of the affected stocks, to a degree varying somewhat in different crosses. One extracted

stock of homozygous R^r: Cornell gave reasonably good seed-color expression in F_1's with several of the group *D* alleles, and these F_1's were used in this study. The F_1 cultures tested for mutation frequency, which are reported in Table IV, were produced by crossing plants homozygous for the various group *D* alleles by a single

TABLE IV. FREQUENCY OF SEED-COLOR MUTATION OF *R* IN HETEROZYGOTES VARIOUS R^r AND R^g ALLELES VS. R^r: CORNELL

Genotype	Mutation Frequency	Mutants		R^r: Cornell Mut. Rate*	L. E.
		r^r	r^g		
				($\times 10^{-4}$)	
R^r: C/R^r: Pampa	3/19,812	3	0	3.0	8.9 – 0.6
R^r: C/R^r: Winnebago	10/29,607	7	3	4.7	9.7 – 1.9
R^r: C/R^r: Chickasaw	1/13,280	1	0	1.6	8.4 – 0.0
R^r: C/R^r: Deaurle	1/11,248	1	0	1.8	9.9 – 0.0
R^r: C/R^g: Valentine	1/18,765	1	0	1.1	5.9 – 0.0
Total	16/92,712	13	3	2.8	4.8 – 1.5

* Number of mutants to r^r one-half of the number of gametes tested.

plant of homozygous R^r: Cornell. An R^g allele, R^g: Valentine, derived from one of the Indian accessions, was also included. The ears produced by the F_1 cultures of the group *D* stocks, pollinated *en masse* by r^g, included some ears of rather variable seed-color, but only a very small proportion in which seed color was too variable to permit examination for colorless mutants. Among the apparently mutant seeds, completely free of aleurone color, several were found on genetic test to be *R r*, indicating that the absence of aleurone color in the seed was due not to mutation of *R* to *r*, but to the failure of appreciable aleurone color development by the *R* concerned. In every case, these plants showed the group *D* phenotype, showing that the weak aleurone color development was associated with the group *D* allele.

No colorless seed mutants with plant-color characteristic of group *D* were found. Of the 17 seed-color mutants identified, 14 were r^r, with plant-color effect similar to those of R^r: Cornell. These may be ascribed to mutation of the R^r: Cornell allele. The other three, all

of which occurred in the *R*^{r}: Cornell/*R*^{r}: Winnebago cultures, were typical *r*^{g} mutants, devoid of anthocyanin at all stages of development. These three mutants were clearly distinguishable, in the seedling stage of development, from their *R*^{r}: Winnebago sibs, which showed good anthocyanin coloration in the coleoptile, and some anthocyanin coloration in the epicotyl and leaf sheath. Each of the three *r*^{g} mutants produced a self-progeny in which the *r*^{g} phenotype was confirmed. One of the mutants was crossed with both *r*^{g} *V*^{r} and *r*^{g} *S*^{r}. The specific modifiers *V*^{r} and *S*^{r} intensify the action of the group *D* alleles including *R*^{r}: Winnebago, resulting in distinct anthocyanin pigmentation of a number of tissues of the mature plant. They show no effect upon anthocyanin pigmentation of *R*^{g} and *r*^{g} plants. The F_1 progenies produced by crossing the *r*^{g} mutant with *r*^{g} *V*^{r} and *r*^{g} *S*^{r} were also devoid of anthocyanin. The possibility that the three *r*^{g} mutants were the result of mutation of the *R*^{r}: Cornell parental allele cannot be excluded, but the result suggests the possibility that seed-color and plant-color mutation are not independent in *R*^{r}: Winnebago. The evidence of independent occurrence of seed-color and plant-color mutation at present is derived wholly from alleles of groups *A* and *B*.

The data of Table IV also extend the evidence of modifier effects upon the frequency of *R*^{r}: Cornell mutation. In all of the F_1's tested, the calculated frequency of mutation of *R*^{r}: Cornell gametes (number of *r*^{r} mutants/half the number of F_1 gametes tested) is substantially lower than that observed in the original Cornell stock.

Mutation Frequency in Extracted Homozygous Stocks

Systematic determination of mutation rate in F_2 and F_3 derivatives have been made in the *R*^{r}: Cornell/*R*^{g}: Valentine cross, and to a very limited extent in a few other crosses. In addition, in a number of cases in which *R*^{r}: Cornell was crossed with a phenotypically dis-

tinguishable R allele, or with an r allele, homozygous R^r: Cornell derivatives have been extracted and tested for mutation frequency.

The R^r: Cornell/R^g: Valentine cross used in the study of F_2 and F_3 derivatives was not that reported in Table IV, but was an earlier cross of the original Cornell stock with R^g: Valentine. Three F_1 plants were selfed to produce F_2 cultures, which showed substantially equal mutation frequencies, totaling 17/65,955. The F_3 cultures were grown from self-progenies of 2 R^r R^r, 9 R^r R^g, and 11 R^g R^g plants of the F_2. The totalled mutation frequency in F_2 and F_3 cultures from selfed R^r R^g plants was 30/118,090, the mutations including 27 to r^r and 3 to r^g. Since the total number of R^r and R^g gametes tested in these cultures was approximately equal, it is evident that the higher frequency of mutation of R^r: Cornell is maintained in the hybrid progeny. Of the 3 r^g mutants observed, one occurred in an R^g R^g plant, and therefore was certainly due to mutation of R^g: Valentine. In addition, the totalled F_3 progenies from R^g R^g plants yielded one r^g mutant among 87,714 gametes tested. It is clear therefore that the R^g allele gives seed-color mutations, but the maximum estimate of its mutation frequency in this experiment, assuming that all the r^g mutants observed were from mutation of the R^g allele, was 4/146,759. (The estimated number of gametes tested, as here given, is equal to one-half of the tested gametes in the F_2 and F_3 cultures from R^r R^g plants, plus all of the tested gametes in the F_3 cultures from R^g R^g plants). This represents a mutation rate of 0.27×10^{-4}.

The mutation frequency in homozygous R^r: Cornell plants was determined for an adequate sample of gametes in only one F_3 progeny of an R^r R^r plant. The frequency in this case was 4/19,553 (rate 2.0×10^{-4}). In addition the three F_2 cultures tested each included approximately 5000 tested gametes of homozygous R^r R^r plants. The mutation rate of R^r: Cornell determined in the R^r R^r plants of these three cultures were 6.1, 3.5, and

3.5 ($\times 10^{-4}$) respectively. Thus the data give no indication of reversion to the high rate characteristic of the Cornell parent, but the number of cultures tested is obviously too small to make the observation significant as to the type of modification involved.

Homozygous R^r: Cornell cultures extracted from crosses with miscellaneous stocks vary widely in mutation frequency, as shown in Table V. The extracted stocks listed are derived from crosses of R^r: Cornell with miscellaneous stocks with distinguishable R alleles.

TABLE V. FREQUENCY OF SEED-COLOR MUTATION IN EXTRACTED STOCKS OF R^r: CORNELL

R^r: Cornell Extraction	From Cross With:	Mutation Frequency	Mutation Rate ($\times 10^{-4}$)	L. E.
A	R^r: Arrow	14/27,255	5.1	8.6 – 2.8
C	R^r: Catspaw	7/12,431	5.6	11.6 – 2.3
Ch	R^r: Chickasaw	27/17,455	15.5	22.6 – 10.2
V	R^g: Valentine	4/19,553	2.0	5.2 – 0.6
W 1	R^r: Winnebago	14/24,630	5.7	9.5 – 3.1
W 2	"	35/39,535	8.9	12.3 – 6.2
W 3	"	5/13,182	3.8	8.9 – 1.2
W 4	"	15/17,071	8.8	14.4 – 4.9
Y	r^r: Yankee	1/11,860	0.8	4.7 – 0.0

Extraction Y is from a cross of R^r: Cornell with an r^r stock, and the data represent the frequency of mutation in the R^r R^r plants of the F_2. The other extractions are from crosses with various R stocks. In each case the extracted stock is derived from the self-progeny of a single homozygous R^r: Cornell plant of the F_2 or F_3 generation. The extractions designated W-1, W-2, W-3, and W-4 were extracted from four different F_2 plants of the Cornell-Winnebago hybrid.

The mutation rates in these homozygous R^r: Cornell stocks range from a level almost equal to that of the original Cornell stock to rates of $1–2 \times 10^{-4}$. In general the mutation rates of these homozygous R^r: Cornell stocks, in which about 50% of the residual genotype has been replaced by other genotypes at random, are similar

to those found for other R^r alleles of group *A*, as shown in Tables I and II.

Considering the results from both heterozygous and homozygous R^r: Cornell cultures, it is evident that the unusually high mutation frequency in the original Cornell stock is not due to any intrinsic instability of the R^r: Cornell allele. It is due at least in large part to the presence of mutation-favoring genes in the Cornell stock. In crosses with other stocks at random the mutation rate is commonly much reduced. The mutation-favoring effect of the modifier complex characteristic of the Cornell stock was recessive in all of the crosses in which R^r: Cornell mutation frequency could be determined in the F_1. Homozygous R^r: Cornell cultures of sharply reduced mutation frequency were readily obtained, though crosses with only a rather small number of stocks were tested, and though these were necessarily stocks unselected as to mutation-modifier genotype. It is reasonable to suppose that with systematic selection and repeated backcrossing to stocks of low-mutation genotype, a stock of R^r: Cornell of high stability could be extracted.

Comparative Effects of Modifier Genotypes upon Seed-Color and Plant-Color Mutation

Each of the derivative stocks listed in Table V represents a modifier complex of known effect upon seed-color mutation frequency of R^r: Cornell. Do the modifier complexes which sharply reduce seed-color mutation frequency similarly reduce plant-color mutation frequency of the same allele? It would be interesting to know the plant-color mutation frequency of R^r: Cornell in each of the extracted cultures, to determine the relative effect of each modifier genotype upon the two types of R^r mutation. Because of the technical difficulty of determining plant-color mutation frequency in significant populations this was not feasible, but determinations of plant-color mutation frequency were made in all of the cultures tested in the four extractions from the cross with R^r:

TABLE VI. EFFECT OF VARIOUS MODIFIER GENOTYPES ON SEED-COLOR AND PLANT-COLOR MUTATION RATE OF *Rr*: CORNELL

Rr: Cornell Stock	Pedigree	Seed-Color Mutation			Plant-Color Mutation		
		Frequency	Rate	L. E.	Frequency	Rate	L. E.
Extr. W-1							
54-1257	1-1 (×)	8/12,550	6.4		12/ 8,807	13.6	
1258	1-2 (×)	6/12,080	5.0		1/ 3,002	3.3	
Total		14/24,630	5.7	9.5 – 3.1	13/11,809	11.0	18.8 – 5.9
Extr. W-2							
59-138A	2 (×)	3/ 3,306	9.1		0/ 2,670	0	
132A	2-1 (×)	2/ 2,877	7.0		0/ 2,332	0	
132B	2-2 (×)	1/ 6,083	1.6		0/ 5,056	0	
133A	2-3 (×)	8/ 7,479	10.7		4/ 6,249	6.4	
133B	2-6 (×)	1/ 2,193	4.6		0/ 1,856	0	
61-1220	2-3-1 ×6	7/ 9,412	7.4		2/ 7,924	2.5	
1221A	2-3-11 ×6	9/ 5,336	16.9		0/ 3,576	0	
1221B	2--3-6 (×)	4/ 2,849	14.0		0/ 1,882	0	
Total		35/39,535	8.9	12.3 – 6.2	6/31,545	1.9	4.1 – 0.7
Extr. W-3							
59-138B	4 (×)	3/ 3,172	9.5				
136A	4-7 (×)	0/ 6,264	0		0/ 2,417	0	
134D	4-9 (×)	2/ 3,746	5.3		0/ 5,281	0	
					0/ 3,248	0	
Total		5/13,182	3.8	8.9 – 1.2	0/10,946	0	3.4 – 0
Extr. W-4							
59-138C	7 (×)	7/ 9,135	7.7		0/ 6,346	0	
137A	7-1 (×)	5/ 4,862	10.3		1/ 3,922	2.5	
137B	7-3 (×)	3/ 3,074	9.8		1/ 2,136	4.7	
Total		15/17,071	8.8	14.4 – 4.9	2/12,404	1.6	5.8 – 0.2
For Comparison: Original Stock		41/23,204	17.7	23.9 – 12.8	4/13,053	3.1	7.8 – 0.8

Winnebago. The results are shown in Table VI, together with the seed-color mutation frequency in the same cultures, and with the total seed and plant-color mutation data for the pure Cornell stock for comparison.

The four derivative stocks represent extractions from four F_2 plants of homozygous R^r: Cornell constitution, all of which occurred in the self-progeny of a single F_1 plant. The relationship of the various cultures is indicated in the column headed "Pedigree." The pedigrees as given represent the plant numbers of the individuals used in each generation, beginning with the F_2 of the R^r: Cornell $\times$ R^r: Winnebago cross. Thus, for example, under Extr. W-2, all of the pedigrees begin with "2," since all of the cultures are derived from plant number 2 of the F_2 culture. Culture 59-138*A* was an F_3 culture grown from seeds of the self-progeny of this individual, and is therefore represented as 2 (x). Cultures 59-132*A*, 132*B*, 133*A* and 133B were F_4 cultures, grown from seeds of four selfed plants of this F_3, namely 2-1, 2-2, 2-3 and 2-6. Cultures 61-1220, 1221*A* and 1221*B* were F_5 cultures, grown from seeds of selfed or sibbed plants of culture 59-133*A*, as indicated by the individual pedigrees.

The mutation rates, both for seed-color and for plant-color mutation, differ considerably among the cultures within the various extractions. This is to be expected, since even with a very small number of modifying factors involved, segregation for these might well occur within the inbred progeny of a single F_2 plant.

Significant evidence on the degree of correlation between seed-color and plant-color mutability among plants of varying modifier genotype is very difficult to obtain, because of the large sampling error in all determinations of mutation frequency. For example, suppose that in the hybrid progenies here considered plant-color mutation rates were actually affected by a modifier system wholly independent of that affecting seed-color mutation frequency. The seed-color mutation rate, in almost all of the extracted cultures, is reduced well be-

low the level found in the Cornell stock. If plant-color mutation rate were reduced in still greater proportion in some of the extractions, it would be practically impossible to secure significant evidence of the fact. Most of the extractions tested gave no plant-color mutations, but none of these determinations is significantly below a rate of 5 ($\times 10^{-4}$), while the rate determined in the original Cornell stock is only 3.1. The demonstration of a plant mutation rate in an extracted culture, significantly lower than 1.3×10^{-4} (a rate reduced below that of the Cornell stock in proportion to the average reduction in seed-color mutation rate), would require a test of at least 30,000 seedlings of the culture concerned. But even this would not constitute significant evidence of disproportionate mutation rates, for the standard rate determined for the original stock is itself based on only four mutations, and therefore has a large sampling error. Consequently, the only practical possibility of finding significant discrepancies in plant-color vs. seed-color mutation rate is in extractions with the plant-color rate so greatly increased as to be significantly above that of the Cornell stock.

The extracted culture, 54-1257, represents a case of this kind. Its mutation frequency, in comparison to that of the Cornell stock, was as shown below:

	Seed-Color Mutation			Plant-Color Mutation		
	Frequency	Rate	L. E.	Frequency	Rate	L. E.
Cornell stock	41/23,204	17.7	23.9 – 12.8	4/13,053	3.1	7.8 – 0.8
54-1257	8/12,550	6.4	12.6 – 2.7	12/ 8,807	13.6	23.8 – 7.0

Thus the seed-color mutation frequency of R^r: Cornell in the extracted culture was obviously significantly lower than in the Cornell stock, while its plant-color mutation frequency was apparently significantly higher. The significance of the difference in plant-color mutation rate may be determined as follows*:

If the probability of mutation is the same in both pop-

* I am indebted to Dr. W. L. Stevens for suggesting the method of determining significance here used.

ulations, the 16 plant-color mutations observed should be distributed between the two populations in proportion to the number of gametes tested, except for the effect of sampling fluctuations. The expected number of mutants in the Cornell stock (population 13,053) is 9.55; in the extracted culture (population 8,807) 6.45. Is the observed distribution of 4:12 a significant deviation from the expected distribution of 9.55:6.45? The probability of a frequency as high as 9.55 in the Cornell stock population, among 16 mutants in the total of the two populations, may be determined from Stevens' table, taking $n = 4$, $N = 16$, $p = n/N = 0.25$. The upper limit of the expectation at the $P = 0.05$ level is 8.39. At the $P = 0.01$ level, the upper limit of the expectation is 9.59, approximately equal to the value expected on the hypothesis of equal mutability in the two populations. Thus the difference is clearly significant. The R^r: Cornell gene in the extracted culture mutates in seed-color effect distinctly less frequently, and mutates in plant-color effect distinctly more frequently, than in the original Cornell stock.

There is no evidence of a general rise in plant-color mutation rate, accompanying the general reduction in seed-color mutation rate, or of any negative correlation between seed-color and plant-color mutation rate. Three cultures with significantly reduced seed-color rate, each tested for plant-color mutation in more than 5,000 gametes, gave no plant-color mutations.

Comparing the extracted cultures with one another yields some additional contrasts of interest, although most of the individual populations are too small for significant comparison. All of the extracted cultures are homozygous for the single R^r: Cornell gene which was present in the F_1 individual from which the extractions were derived. There is evidence of segregation within Extr. W-2 in the comparison of the progenies of the F_3 plants 2-2 and 2-3. The self-progenies of these two

plants, tested in cultures 59-132*B* and 133*A*, differed widely in seed-color mutation rate (rates 1.6 *vs.* 10.7), but the difference was not statistically significant. The progeny cultures of 2–3, cultures 61–1220, 1221*A*, and 1221*B*, confirmed its relatively high mutation rate. The totaled data for the four cultures derived from plant 2-3, which may be grouped as Extr. W-2-3, showed a seed-color mutation rate of 11.2, based on 25,076 tested gametes. This differs significantly from that of culture 59-132*B*. The data for the cultures grouped as Extr. W-2-3 are of interest also in comparison to the data for culture 54-1257. They show a significantly lower plant-color mutation rate, and a higher, though not significantly higher, seed-color mutation rate.

The data indicate that the modifier system affecting plant-color mutation rate is in some degree independent of that affecting seed-color mutation rate.

Mutation Frequency as an Indicator of Allelic Differences

The experiments on allelic variability previously mentioned (Stadler and Fogel, 1943, 1945), which will be reported in detail shortly, indicate that many *R* alleles of similar phenotypic effect are distinguishable by minute phenotypic differences. These experiments suggest that, among alleles of a given gene extracted from relatively unrelated populations, allelic diversity may be the rule rather than the exception. The appearance of uniformity may be due only to the insensitive criteria of phenotypic effect by which we recognize allelic differences.

May mutation frequency be used to supplement phenotypic effect as a criterion of allelic identity? If so it might be possible to recognize differences in gene constitution which are beyond the limit of phenotypic detection, or perhaps differences in gene constitution which have no effect upon gene action.

The experiments reported above give little support to

this hope. They show that there are actual differences in mutability of different alleles of *R*, but they also show that the effect of modifiers upon the mutation rate is far too great to permit valid comparison of the mutation rates of alleles in different races or even in related cultures. With more definite knowledge of the modifier action, it may be possible ultimately to compare phenotypically indistinguishable alleles in cultures equalized as to mutation modifiers. For the present, a direct comparison in mutability can be made only between pairs of alleles in heterozygous plants. Such comparisons are possible only between alleles which yield phenotypically distinguishable mutants. The comparison in mutability is thus limited to alleles already known to be distinct.

One of the experiments on mutation of *R* in different races and their F_1 hybrids, however, gave results suggesting a difference in mutational behavior between alleles identical or very similar in phenotypic effect. The result is not conclusive, but is here reported as a background for further studies now in progress.

The alleles involved are R^r: Quapaw and R^r: Cornell. Both are members of group *A*, and were included among the sample of 22 R^r alleles studied in the gene variability experiments. The plant-color effect of the group *A* alleles is shown in the same tissues by all of the alleles studied, the intensity of the pigmentation varying rather widely with environmental conditions and with genetic modifiers. The evidence of phenotypic differences between the R^r alleles of group *A* comes from comparison of the plant-color phenotype in sib plants in progenies in which one R^r allele is compared with the r^r mutant of another. For example, in the progeny of the cross R^r: Boone/r^r: Black $\times$ r^g (or the cross r^r: Boone/R^r: Black $\times$ r^g), all of the plants are heterozygous for *R*-determined plant color due to the Boone or Black allele. They may be examined objectively for minor differences in plant-color expression, and the classification may later be checked by seed-color identification. In these objective

tests it is found that the plants of the two contrasted types may be identified, not with complete accuracy but certainly with better than accidental correctness. Separations successful according to this standard may be made with the alleles in all of the combinations necessary to establish an order of decreasing intensity. It appears that at least six and probably all seven of the group *A* alleles tested in this way are distinguishable. The possibility that the differences in phenotype are due to closely linked modifiers is not excluded. In this seriation R^r: Cornell and R^r: Quapaw occupy adjoining positions, R^r: Cornell showing slightly more intense pigmentation than R^r: Quapaw.

The Quapaw stock and the Cornell stock have both been tested rather extensively for seed-color mutation rate (see Table I), and both yield mutations at rates high enough for quantitative comparisons. In addition to the various F_1's of Cornell with stocks carrying distinguishable *R* alleles, which have been reported, an F_1 of the Cornell stock crossed with the Quapaw stock was tested for mutation rate. In this F_1, as in the other F_1's tested, the mutation rate was reduced below half the rate characteristic of the Cornell stock. This result indicates that the stock crossed with Cornell (in this case the Quapaw stock) brought into the F_1 dominant modifiers or modifier complexes materially reducing the mutation rate of the R^r: Cornell gametes tested.

Now, if the gene designated R^r: Quapaw is identical with the gene designated R^r: Cornell, the presence of such modifiers in the Quapaw stock should have similarly reduced the mutation rate in that stock. The mutation rate in the F_1 was not only lower than half the rate in the Cornell stock, but lower than half the rate in the Quapaw stock. Thus the R^r: Quapaw gametes tested in the F_1 also mutated at a lower rate than in the parental stock, presumably due to modifiers reducing R^r: Quapaw mutation, which were brought into the F_1 by the Cornell parent. The same considerations apply to these fac-

tors; if R^r: Cornell is the same as R^r: Quapaw, why do not these factors similarly reduce mutation rate in the Cornell stock?

The mutation data on the two F_1 cultures and on the four cultures of each of the parent stocks included in the same planting for comparison are shown in Table VII.

The mutation rate in the F_1 is clearly below that in either parent, and the difference is highly significant ($P < 0.01$) in both comparisons. Assuming that all three mutants in the F_1 are from R^r: Cornell, and thus crediting R^r: Cornell gametes with a mutation frequency of 3/14,724 in the F_1, the assumed mutation rate for R^r: Cornell gametes in the F_1 is 2.0, instead of 19.7, as in the Cornell stock. The difference is highly significant ($P < 0.01$). Assuming similarly that all three mutants in the F_1 are from R^r: Quapaw gametes, the reduction in frequency as compared to the Quapaw stock is on the borderline of significance at the $P = 0.05$ level.

The Quapaw parent plants used in making the cross were two plants of culture 45-1801. This was the progeny of a cross of two sib plants grown from seeds of the sample received originally from Mr. Kempton, who had maintained the stock of four consecutive generations of sib pollination. The sample furnished consisted of the seeds produced by a single sib pollination. Ten of these seeds were planted as culture 41-239, and plants *5* and *6* of this culture were crossed to produce the seed from which culture 45-1801 was planted. The four cultures tested to determine mutation frequency in the Quapaw stock were the self-progenies of four other plants grown from seeds of the original sample, in a later planting (culture 49-208*B*). Thus the Quapaw parent of the F_1 is descended from two plants of the fourth generation inbred, while the Quapaw selfs are descended from four other plants of this culture. It might be supposed that the plants of this inbred culture were segregating for factors sharply reducing the mutation frequency of R^r: Quapaw, and that these chanced to be present in the plants from which

TABLE VII. Frequency of Seed-Color Mutation of *R* in Quapaw Stock, Cornell Stock, and F_1

	Culture	Parentage	Mutation Frequency	Mutation Rate	L. E.
Quapaw Stock	51-1041	49-208 B-4 (×)	10/ 8,665	11.5	21.2 – 5.5
	1156	208 B-3 (×)	3/ 4,098	7.3	21.4 – 1.5
	1157, 58	208 B-6 (×)	3/11,360	2.6	7.7 – 0.5
	1159	208 B-8 (×)	2/ 2,595	7.7	27.8 – 0.9
Total			18/26,718	6.7	10.7 – 4.0
Cornell Stock	51-1036	49-233-1 (×)	12/ 5,338	22.3	39.3 – 11.6
	1145	45-1809-8 (×)	5/ 2,195	22.8	53.1 – 7.4
	1146	45-1809-11 × 8	10/ 5,320	18.8	34.6 – 9.0
	1147	49- 18-1 (×)	7/ 4,370	16.0	33.0 – 6.4
Total			34/17,223	19.7	27.6 – 13.7
Quapaw/Cornell F_1	51-1143	45-1809-10 × 1801-2	1/ 8,027	1.2	6.9 – 0.0
	1144	45-1811- 3 × 1801-9	2/21,420	0.9	3.4 – 0.1
Total			3/29,447		

the parent of the F_1 was descended and not in any of the four plants selfed to represent the Quapaw stock. Another F_1 between the Quapaw stock and the Cornell stock was therefore tested for mutation frequency. The Quapaw parent in this case (culture 56-917) was a third generation descendant (by self and sib pollination) from two additional plants grown from seeds of the original sample (culture 41-239, plants *1* and *4*). The mutation data for the second F_1 were as follows:

Mutation Frequency	Mutation Rate	L. E.
2/45,822	0.4	1.6 – 0.1

The second F_1 confirms the low mutation rate observed in the first F_1, and the differences in mutation rate in comparison to the parent stocks are even more clearly significant.

It seems probable therefore that the R^r: Quapaw allele differs from the R^r: Cornell allele in its reaction to modifiers of mutation frequency. This is not positively established by the evidence, for it is possible that some complementary action of modifiers may be involved, such as to make the modifier genotype in each stock relatively ineffective except in combination with the modifier genotype from the other.

The prospect of distinguishing critically between these possibilities by further study of the progeny of this hybrid is slight. It would not be possible in the hybrid progenies to distinguish the two R^r alleles from one another, or to distinguish their mutants from one another. Nor would it be possible to identify the mutation modifiers individually by linked marker genes, except by introducing a new complex of mutation modifiers with each linkage tester stock used.

A more promising approach is to attempt, in separate experiments with well-marked stocks, to identify individual modifiers of R^r: Cornell mutation, and then to test these modifiers for their effect upon R^r: Quapaw mutation. The use of the R^g mutants of R^r: Cornell and

R^r: Quapaw may also make possible comparisons of Cornell and Quapaw response in the same plants, although the reaction of the mutant R^g alleles to the mutation modifiers cannot be predicted in advance of the evidence. The seed-color mutation rate of the mutant allele R^g Cornell is sharply reduced below that of the parent allele R^c: Cornell (Stadler 1946), and the possibility of changed reaction to modifying factors incidental to this gene alteration is a problem of interest in itself. The apparent independence of variations in seed-color mutation rate and plant-color mutation rate in the same R^r gene, reported in this paper, indicates that some, at least, of the modifiers of R mutation are quite specific as to the type of gene alteration affected. It would be desirable therefore to test the same individual modifiers of R^r: Cornell seed-color mutation not only for effects on seed-color mutation of R^r: Quapaw, but for effects on seed-color mutation of R alleles of the other groups, on seed-color mutation of R^g: Cornell, and on plant-color mutation of R^r: Cornell.

If individual mutation modifiers may be identified, the systematic determination of their effects upon the various R alleles and the various types of R mutation might provide a new technic for the analysis of allelic relations. Some progress has been made in the identification of individual modifiers of seed-color mutation of R^r: Cornell. This will be reported in the next paper of this series.

Summary

1. The frequency of seed-color mutation differs widely in different races. The highest rate found was 18.2×10^{-4} in an inbred race designated "Cornell stock." Other stocks gave lower rates, including several with no mutations in populations of 10,000 to 20,000 tested gametes.

2. Plant-color mutation rates, determined on a smaller scale, also vary rather widely. There appears to be no correlation between frequency of plant-color mutation and frequency of seed-color mutation in the same alleles.

3. Differences in seed-color mutation frequency are due in part to intrinsic differences in mutability of the various *R* alleles. *R* alleles which yield seed-color mutants distinguishable by their plant-color effects may be compared in mutation rate under strictly comparable conditions in heterozygotes. In these trials significant differences in mutation rate for the two alleles compared are found in some cases.

4. Seed-color mutations in such heterozygotes marked also at the *g* locus (proximal to *R* at about 14 cross-over units) are not associated with crossing over in the *R-g* segment. Among the 14 r^r and r^g mutants tested, only two showed crossing over in this segment.

5. Differences in mutation rate between different races are also due in large part to the effects of modifying factors. In F_1 hybrids produced by crossing the Cornell stock with various other stocks, the R^r: Cornell gametes of the F_1 commonly yield mutations at a rate much lower than that found in the Cornell stock.

6. Homozygous R^r: Cornell derivatives of lowered mutation frequency are readily extracted from crosses of the Cornell stock with strains carrying distinguishable *R* alleles. Among extracted stocks of various R^i alleles, R^r: Cornell stocks are not outstanding in mutation frequency.

7. The varying modifier genotypes occurring in different homozygous R^r: Cornell extractions also sharply modify plant-color mutation rate, apparently independently of their effect upon seed-color mutation rate.

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STEVENS, W. L., 1942. Accuracy of mutation rates. *Jour. Genetics,* 43: 301–307. 1945. In Fisher, R. A., and F. Yates, Statistical Tables for Biological Agricultural and Medical Research, Edinburgh, 1945, Table VIII.

AN APPLICATION OF THE ALLOMETRY EQUATION TO THE STUDY OF GROWTH IN *CALLINECTES SAPIDUS* RATHBUN

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SEVERAL investigators have demonstrated the application of the allometry equation $Y = a X^b$ to problems dealing with the relative growth of a body part in relation to the whole body (Huxley, 1932; Miller and Hoy, 1939; Newcombe, Sandoz and Rogers-Talbert, 1949; Newcombe, Campbell and Eckstine, 1949). The application of this relative growth equation to a set of data is dependent upon the existence of a straight line relationship when the data are plotted on a double logarithmic grid. The growth coefficient b expresses the ratio of the percentage growth rates of the dimensions being studied and may be obtained by measuring the slope of the line through the logarithmic plotting by inspection or else by the method of least squares which is used in this paper. The constant a indicates the ratio

$$\frac{Y}{X^b}$$

and has been called the "initial growth index" (Huxley and Teissier, 1936).

Growth studies of the Blue Crab, *Callinectes sapidus* Rathbun in the Chesapeake area by Newcombe, Sandoz and Rogers-Talbert (1949), conducted at the Virginia Fisheries Laboratory, have shown that as the crab grows it changes in form; hence there are disproportionate rates of growth in respect to the several linear dimensions studied. The dimensions examined include: width (W), the shortest distance between the ends of the 9th pair of antero-lateral spines of the carapace; length (L), the perpendicular distance across the carapace from a point im-

mediately posterior to the rostrum to a point just above the first segment of the abdomen; eye to spine (E), the distance between the first and ninth antero-lateral spines of the right margin of the carapace; the propodus (C) of the right chela represented by its maximum length; and the distance between the preorbital spines (interocular width) F (figure 1).

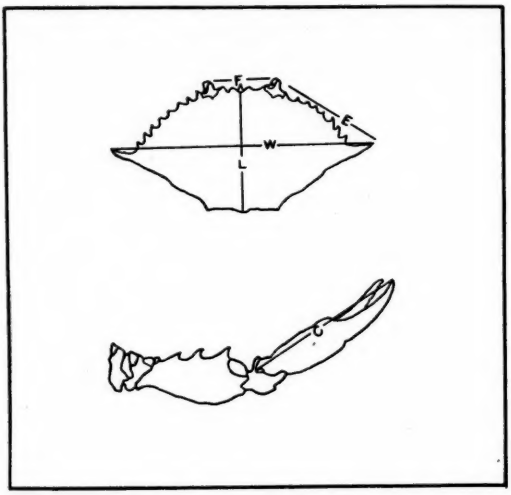

FIG. 1. Outline drawing of the Blue Crab, *Callinectes sapidus* Rathbun, showing the dimensions measured. W = width; L = length; E = distance between the 1st and 9th antero-lateral spines of the right margin of the carapace (eye to spine distance); C = length of the propodus of the right cheliped; and F = distance between the preorbital spines (interocular width). (after *Newcombe, Sandoz* and *Rogers-Talbert*, 1949).

In addition to a description of several growth dimensional ratios, the number of moults was estimated by direct use of a curve obtained by plotting a series of initial and final width measurements, that is, widths before and after shedding (figure 2). Knowing the average width of the first instar to be 2.47 mm, obtained by measuring large numbers of first stage post-larval crabs reared from megalops by my associate, Mildred D. Sandoz, it was

readily possible to estimate from the curve the width of the different instars and their approximate number (Newcombe, 1949).

In this paper interest centers on the application of a

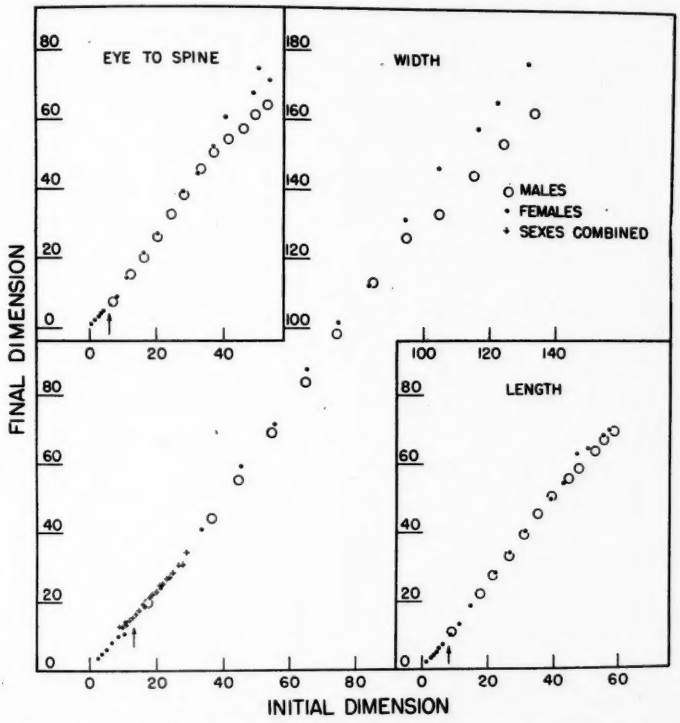

FIG. 2. Relation of initial to final dimensions (before and after moulting) in *Callinectes sapidus* Rathbun. Sexes combined in groups to the left of the arrows and in the plus groups, the latter serving as a check on the former in regard to slope of line of best fit. (From *Newcombe, Sandoz* and *Rogers-Talbert,* 1949).

method for analyzing the intensity of dimensional growth throughout the size range of the species. Also, there is provided a mathematical basis for determining the theoretical number of instars characteristic of the species which may be extended to related forms. For these pur-

poses, the allometry formula has been employed (Brody, 1945 p. 608; and Huxley, 1932). The application of this parabola to the data presented by Gray and Newcombe (1938a, 1938b) and Newcombe *et al.* (1949) together with the information about relative growth rates thereby revealed, is discussed here.

Acknowledgement is made to Professor P. S. Dwyer of the Statistical Laboratory of the University of Michigan for most generous and helpful counsel.

Results and Discussion

Huxley's applications of the allometry equation to diverse types of growth data have contributed greatly to the meaning of differential growth processes. He recognized *heterogonic growth* and *isogonic growth* depending upon whether the rate of growth of a body part is different from or similar to that of the body. In this analysis the problem is whether or not the rate of growth of a particular dimension, e.g. width, remains the same throughout life. The percentage increment of width in the case of a crab 20 mm wide may be 32, and that of one 80 mm wide may be the same or it may be different. Hence, it is desirable to obtain a mathematical expression of the size range or ranges over which the percentage increments are the same as well as to establish the sizes at which proportionality and rates of growth undergo changes.

The data on which these analyses are based are from Gray and Newcombe (1938b); and Newcombe *et al.* (1949) (figure 2). By plotting the logarithms of the initial dimensions against the logarithms of the final dimensions (measurements before and after moulting), it has been possible to break down the growth curve into parts or "stanzas" that have similar growth rates and to correlate the size at which a break occurs with related growth or physiological behavior (figure 3). The constants of the allometry equations established from these data may be

used as a basis for measuring the significance of possible differences in growth rates of the same dimension in different parts of the size range or of different dimensions within the same size range of the species.

Growth rates of linear indices of body size. Growth

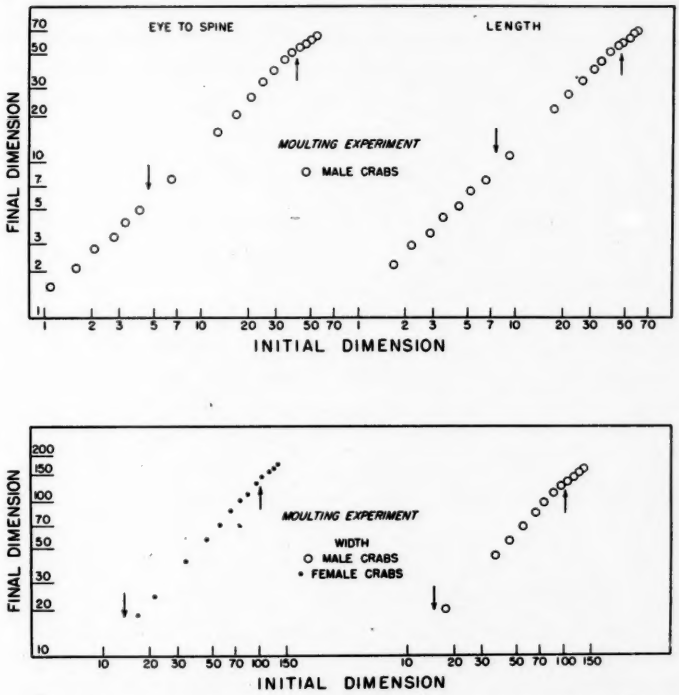

FIG. 3. Growth trends for width, length and eye to spine dimensions as shown by plotting initial size (before moulting) against final size (after moulting) on a double logarithmic scale.

rates of individual linear indices of body size during the different moultings are in some instances described by a single straight line on a double logarithmic grid. In others, displacements are clearly evident and two or even three straight lines are required (figures 2 and 3). The changes may be abrupt, i.e. during one moult, even though

the regression indicates a gradual change. For example, an individual female crab becomes sexually mature in its last instar but that stage is not reached at the same size in all crabs, hence the composition of the data yields a curvilinear regression. A comparison has been made of the b values for the W, L, E, F, and C dimensions in the group range I–VII (table I). The difference between the values of b for L and C, namely 0.93 and 0.79 was found to be significant at the 5 percent level but not at the 2 percent level. In the remaining nine comparisons of pairs of b values, not a single difference was significant at the 5 percent level.

An index to the growth trend is provided by the data of table I. In groups VIII to XV and XVI, the b values for males and females, respectively, are positively allometric and significantly different at the one percent level. A slight displacement is seen to take place in both sexes at a width of about 95 mm. The slope is greatest in the males in every instance. The least noticeable bend in the line is for length (L) while the most conspicuous one is for the eye to spine distance (E) (figure 3). Weymouth and MacKay (1936) associate a comparable change, interestingly at a similar size, with sexual maturity in *Cancer magister*. The slight bend in the logarithmic plotting, near the upper extremity of the regression, is believed to be an index of sexual maturity in *Callinectes sapidus*. This possibility is discussed by Newcombe *et al.* (1949).

The value of b for width in groups I–VII is 0.854 whereas for groups VIII to XV and XVI, males and females, b values are 1.097 and 1.137, respectively. As a partial check on the value of b, namely 0.854, for the first groups, specimens representative of each millimeter group between widths of 9 and 29 mm were brought to the laboratory and held until they moulted (see plus marks on curve in figure 2). These crabs were subjected to laboratory conditions only for short periods as compared with those of groups I to VII that were reared in the labora-

tory from the megalops stage. However, the a and b values for the series 1.68 and 0.88, respectively, were found to be essentially the same as those of the laboratory reared crabs, namely 1.67 and 0.85 (table I). These data suggest that the factor of laboratory rearing is not sufficiently significant to account for the difference in slope between 0.854 and 1.097 in the males and 1.137 in the females of the next larger group. Also, they seem to indicate that the change in growth increment is a gradual one taking place at widths of around 20 mm.

Considering the character of the growth trend in the upper groups, the allometry equation indicates that the males grow less in proportion to their width than the females beyond a width of about 90–100 mm. The change in allometry indicated by figure 3 is more noticeable in the males. In addition to the above treatment of the data, the upper two parts of the curve have been fitted to a single straight line, b values for males and females being 1.020 and 1.100, respectively. The difference is significant at the 1 percent level.

The application of the allometry equation is equally satisfactory for analyzing the growth rates of the length and eye to spine dimensions. Comparing the b values for length in the different groups, it is seen that the slope is similar and the relationship could be expressed by one equation with $a = 1.263$ and $b = 0.991$ for males and with $a = 1.228$ and $b = 1.006$ for females (table I). These differences fail to show significance. However, in view of our understanding of the changing width increments with increasing moults and on a basis of the arithmetic relations shown in figure 2, we recognize trends similar to but less conspicuous than those pointed out in the discussion of width, the essential difference being one of magnitude. In groups VIII to XV and XVI, the values of b are practically identical, namely 1.029 and 1.049 in males and females, respectively, but on a basis of the figures there is a biologically significant difference in slope from the pre-

TABLE I

CONSTANTS OF DIFFERENTIAL GROWTH RATES OF SEVERAL DIMENSIONS OF *Callinectes sapidus* RATHBUN AS EXPRESSED BY THE EQUATION Y = INITIAL SIZE OF THE DIMENSION I.E. SIZE BEFORE MOULTING AND Y = FINAL SIZE AFTER MOULTING. *t* VALUES FOR DIFFERENCES IN *b* VALUES ARE GIVEN

Dimension	Male Specimens				Female Specimens				♂ and ♀	
	Group	Interval of initial dim.	a	b	Group	Interval of initial dim.	a	b	t	P
W *Width*	I–VII	2.5– 11.0	1.67	0.854	.....	9.0– 29.0	1.68	0.881		
	VIII–XV	17.6– 95.4	0.86	1.097	VIII–XVI	16.8– 94.6	0.75	1.137	3.15	< .01
	XVI–XXII	104.8–153.2	2.31	0.869	XVII–XX	104.5–131.5	2.23	0.896	0.24	> .05
	VIII–XXII	17.6–153.2	1.14	1.020	VIII–XX	16.8–131.5	0.86	1.100	3.56	< .01
L *Length*	I–VII	1.7– 6.5	1.35	0.931						
	VIII–XV	9.2– 44.6	1.14	1.029	VIII–XVI	8.9– 43.2	1.03	1.049	0.29	> .05
	XVI–XXI	47.9– 66.5	1.73	0.909	XVII–XX	47.2– 57.2	11.09	0.451	4.68	< .01
	VIII–XXI	9.2– 66.5	1.33	0.978	VIII–XX	8.9– 57.2	1.03	1.050	1.75	> .05
	I–XXI	1.7– 66.5	1.26	0.991	I–XX	1.7– 57.2	1.23	1.006		
E *Eye to Spine*	I–VII	0.7– 4.1	1.37	0.898						
	VIII–XV	6.5– 37.0	1.04	1.072	VIII–XVI	6.1– 37.0	0.86	1.139	2.32	.02 < P < .05
	XVI–XXI	42.0– 61.5	2.15	0.860	XVI–XX	41.7– 54.0	3.99	0.731	0.55	> .05
	VIII–XXI	6.5– 61.5	1.25	1.003	VIII–XX	6.1– 54.0	0.92	1.113	3.89	< .01
	I–XXI	0.7– 61.5	1.35	0.980						
C *Propodus of chaela*	I–VIII	1.3– 5.6	1.54	0.795						
F *Betw. eye Spines*	I–VIII	1.8– 7.7	1.45	0.876						

ceding group. In the larger group it is likely that the changing logarithmic slopes represent small but biologically signficant changes in the growth rates involved. From a biometrical standpoint the difference between $b = 0.978$ and $b = 1.050$ for groups VIII to XXI and XX, respectively, is not significant at the 5 percent level. However, observation of the data shows that the downward slope for the males is demonstrable.

TABLE II

WIDTHS OF INSTARS OF MALE AND FEMALE BLUE CRABS CALCULATED FROM ALLOMETRY EQUATIONS BY STARTING WITH THE WIDTH OF INSTAR I, NAMELY 2.47 MM. THE SIZES OF INSTARS II–VIII (SEXES COMBINED) WERE OBTAINED FROM THE EQUATION $F.W. = 1.67\ I.W.^{0.854}$, THOSE OF THE REMAINING ONES FROM $F.W. = 0.859\ I.W.^{1.10}$ FOR FEMALES AND $F.W. = 1.14\ I.W.^{1.02}$ FOR MALES.

Instar No.	Instar width		Instar No.	Instar width	
	males	females		males	females
I	2.47	2.47	XI	24.92	20.67
II	3.65	3.65	XII	30.29	24.05
III	5.05	5.05	XIII	36.97	28.41
IV	6.66	6.66	XIV	45.30	34.11
V	8.43	8.43	XV	55.73	41.73
VI	10.30	10.30	XVI	68.85	52.07
VII	12.25	12.25	XVII	85.43	66.45
VIII	14.19	14.19	XVIII	106.4	86.90
IX	17.05	15.90	XIX	133.2	116.7
X	20.57	18.01	XX	167.4	161.4

The values of a and b for the eye to spine (E) dimension over the range of groups I–VII are quite similar, but not the same as those for the length and width dimensions. These dimensions adhere to the allometry equation and for purely prediction purposes may be regarded as similar. The b values of groups VIII to XV and XVI show sex differences that are significant at the 5 percent level while those for groups VIII to XXI and XX show differences at the 1 percent level (table I). The equation for males that covers the entire range is $F.E. = 1.349\ I.E.^{0.980}$

The growth equation may now be used as a means for estimating the theoretical numbers of instars for the species. Thus, starting with an initial width of 2.5 mm, the mean width of the 1st instar, the width of the 2nd in-

star obtained by substitution in the equation given in tables I and II, is 3.65 mm, that of the fourth is 5.05 mm, and so forth. On this basis there appears to be 20 instars, the widths of which are given in table II. Weymouth and MacKay (1936) conducted extensive growth studies on the Pacific Edible Crab, *Cancer magister,* and concluded that in British Columbia waters the males and females passed through 17 and 16 post-larval instars, respectively.

It is of interest to compare the variation in values of a and b. Huxley (1927) compared the growth constants of numerous forms and concluded that a is by far the most variable. Hersh (1931) found that for each sex in Drosophila, a decreased in geometrical progression, as b increased in arithmetical progression demonstrating a uniform relation between the two with definite biological meaning. Throughout the series of a and b values compared here, there is an inverse relation between the magnitude of these two constants. No attempt, however, has been made to evaluate the meaning or uniformity of the relation.

Conclusion

1. The allometric growth formula, $Y = a\ X^b$, is a suitable expression for comparing the rates of growth of the several linear dimensions of the Blue Crab, *Callinectes sapidus* Rathbun.

2. During the first seven or eight post-larval moults the relative growth rates of the five dimensions studied do not differ significantly among themselves except in one instance. Values of the growth constant b for increase in body length and in the length of the propodus of the right chela, namely 0.93 and 0.79, respectively, are significantly different at the 5 percent level but not at the 2 percent level, t being equal to 2.67.

3. The allometry expression indicates that rates of growth of the width and eye to spine dimensions show sex differences that are significant at the 1 percent level,

whereas growth in length is quite similar in males and females.

4. Application of the allometry method suggests that negative heterogeny starts at a width of about 95–100 mm in male crabs. This trend of growth appears to be significant and indicative of the average size at which sexual maturity is reached in male Blue Crabs.

5. The allometry equation is a valuable means for analyzing growth trends in respect to particular body dimensions and also for estimating the theoretical number of instars that characterize a particular species.

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COMPETITION BETWEEN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA MELANOGASTER* IN POPULATION CAGES

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THE work of Timofeef-Resovsky (1933, 1935), L'Heritier and Teissier (1937), Park (1943) and others, has demonstrated the value of experiments on interspecific competition as a means of determining the relative fitness of different species or strains of the same species in different environments. The work of Park was concerned with competition of the flour beetles, *Tribolium confusum* and *T. castaneum*. Under certain conditions, *Tribolium confusum* was the more successful competitor, whereas under altered conditions, *Tribolium castaneum* manifested a superiority. Similarly, L'Heritier and Teissier demonstrated that, under certain conditions, the frequency of the mutant "vestigial" fell rapidly when in competition with the wild type *Drosophila melanogaster*. When conditions were changed, however, the frequency of the mutant increased. The studies of Timofeef-Resovsky dealt with geographic strains of *Drosophila funebris*. Equal numbers of eggs of a known strain of *Drosophila funebris* and of a standard strain of *D. melanogaster* were placed in culture bottles. The total number of eggs was so great that a strong competitive system was established in the ensuing developmental stages. A count of the adults of each species that hatched revealed a marked difference in the viability of strains of different geographic origin.

In this paper, a report is made of preliminary experiments on competition between *Drosophila pseudoobscura* and *D. melanogaster*.

The *Drosophila pseudoobscura* employed were derived from ancestors collected at Pinon Flats and at Mather,

California, and are carriers of three different third chromosome arrangements (Standard, Arrowhead, and Chiricahua). Several strains of each type were used to insure the population being representative of the species. The types of *Drosophila melanogaster* used as competitors were (*a*) wild type (Swedish-*b*), (*b*) white, (*c*) white- miniature- forked and (*d*) a strain of flies with six second chromosome recessives *dp b c pr px sp*. This last strain will be referred to as "all-al" (all minus aristaless) since it lacks the aristaless mutation included in the "all" stock (*D.I.S.* 1947). The population cages used were of the type described by Dobzhansky (1947).

Eight competition experiments were carried out. In the first two, the *D. pseudoobscura* population was a mixture of Mather and Pinon flies. The remaining six were conducted as three series of two experiments each. In one cage, a *D. melanogaster* strain was placed together with Pinon flies, and in a second cage, the same *D. melanogaster* strain was run against Mather flies. Table I indicates the strains of each species used, and the numerical results of the competition experiments. In cages 3–8 inclusive, the initial *D. pseudoobscura* populations consisted of equal numbers of all three inversion types in homozygous condition. In cages 1 and 2, all three types were introduced with many individuals already in a heterozygous state. This was because flies for these cages were taken from other population cages containing *D. pseudoobscura*.

The first count was made after 30 days and all subsequent counts were made after 20-day intervals. In some cases, the flies could be collected by merely attaching a culture bottle to the funnel at one end of the cage. For cages containing "all-al," all food cups were removed because of the reluctance of these flies to leave a cage which contains food. All populations were kept at 25° C. during the course of the experiment.

The change in frequency of *D. pseudoobscura* in each cage is represented in Figure 1. It is clear that a popu-

lation composed of a mixture of Mather and Pinon flies cannot compete successfully against either the wild type or the white strain of *D. melanogaster,* although in the

TABLE I: INITIAL POPULATIONS AND NUMERICAL CHANGES OF D. PSEUDOOBSCURA AND D. MELANOGASTER IN POPULATION CAGES

		Days	pseudo-obscura	melano-gaster
Cage 1	10 Swedish-b *D. melanogaster*	0	990	10
	vs. 990 *D. pseudoobscura* from	30	304	3036
	Mather and Pinon	50	4	5736
Cage 2	10 white *D. melanogaster vs.*	0	990	10
	990 *D. pseudoobscura* from	30	893	1213
	Mather and Pinon	50	228	2941
		70	1	4048
Cage 3	20 w.m.f. *D. melanogaster vs.*	0	980	20
	980 *D. pseudoobscura* from	30	561	589
	Pinon	50	189	1813
		70	5	2312
Cage 4	20 w.m.f. *D. melanogaster vs.*	0	980	20
	980 *D. pseudoobscura* from	30	1746	231
	Mather	50	736	718
		70	125	2235
		90	39	3088
		110	2	2685
Cage 5	20 "all-al" *D. melanogaster vs.*	0	980	20
	980 *D. pseudoobscura* from	30	1098	152
	Pinon	50	2327	470
		70	3009	259
		90	2720	0
Cage 6	20 "all-al" *D. melanogaster vs.*	0	980	20
	980 *D. pseudoobscura* from	30	1429	23
	Mather	50	1287	112
		70	1301	262
		90	1292	214
		110	2418	21
Cage 7	500 "all-al" *D. melanogaster vs.*	0	500	500
	500 *D. pseudoobscura* from	30	977	1253
	Pinon	50	830	1933
		70	867	907
		90	1427	313
		110	1997	12
Cage 8	500 "all-al" *D. melanogaster vs.*	0	500	500
	500 *D. pseudoobscura* from	30	872	1696
	Mather	50	995	274
		70	2106	8

latter case, the elimination is much less rapid (cages 1 and 2). In competition with the white-miniature-forked strain, the Mather population declines more slowly than the Pinon population (cages 3 and 4). Mather flies also

exhibit a superiority over the Pinon flies when the initial population is composed of equal frequencies of *D. pseudoobscura* and the "all-al" strain of *D. melanogaster*, although the Pinon flies seem to be more successful during the first 30 days (cages 7 and 8). When the initial

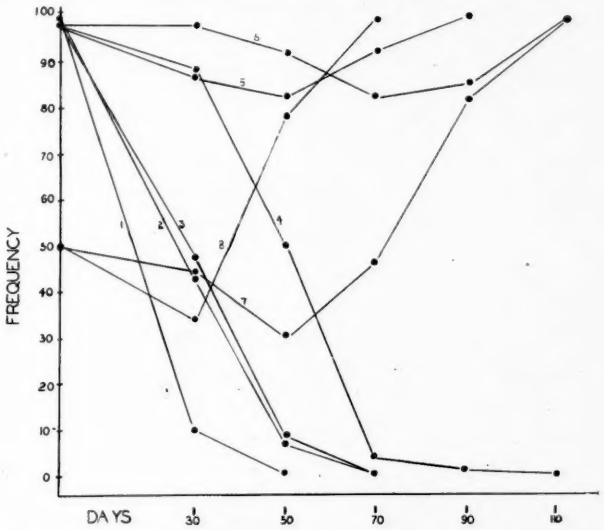

FIG. 1. The changes in frequency of *D. pseudoobscura* in the population cages.

frequency of the "all-al" strain of *D. melanogaster* is low, there is no detectable difference in the competitive abilities of the Mather and Pinon populations (cages 5 and 6). Regardless of its initial density, the "all-al" strain builds up initially, only to be eliminated, no matter with which *D. pseudoobscura* strain it is in competition.

In general, *D. melanogaster* wins when in competition with *D. pseudoobscura*. At the end of 30 days (approximately one and one-half generations of *D. pseudoobscura* and two of *D. melanogaster*), the results obtained when Mather is run against a *D. melanogaster* strain and when Pinon is run against the same strain, show a statistical difference in the competitive values of these *D. pseudo-*

obscura strains. Although each experiment was run only once, the results indicate that the Mather flies are generally more effective competitors with *D. melanogaster* than are the Pinon flies. The initial increase in *D. melanogaster* may be correlated with its tremendous reproductive capacity and shorter developmental period. Reversals similar to those shown in cages 5–8 inclusive, were demonstrated by Park (1948) in competition experiments between species of **Tribolium** and (1941) in intergeneric competition experiments involving **Tribolium, Gnathoceros,** and **Trogoderma.** This points up the complexity of forces which are operating in competition between different species 1 and genera in nature.

Summary

The population cage was successfully employed in studying competition between *D. pseudoobscura* and *D. melanogaster.* Using the wild type, white and white-miniature-forked strains of *D. melanogaster,* this species replaced *D. pseudoobscura.* However, when *D. pseudoobscura* was pitted against the "all-al" strain of *D. melanogaster,* the former species was victorious. An analysis of the populations throughout the course of the experiments suggests that many factors are in operation in interspecific competition.

Acknowledgement: I wish to express my appreciation and gratitude to Prof. Th. Dobzhansky for his guidance throughout the course of this investigation.

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SURVIVAL OF BIRDS AT HIGH TEMPERATURES

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WITH the great expansion of biology in the past century very few of us can truthfully claim to be "naturalists" today. We are taxonomists, geneticists, cytologists, anatomists, morphologists, physiologists, and various other specialists. This compartmentalization creates pitfalls for the unwary. We must remember that our own study is but a small part of biology and strive to work with a due regard for readily available information from other fields. For my own particular interest, birds, this information includes classification and nomenclature, detailed descriptions of external morphology and coloration (including geographic, sexual, and individual variations), distribution and often migration in considerable detail, season of commencing reproduction, nests, egg-shells, plumages of young, and moults. All of this is known for most birds of the temperate zones of the northern hemisphere, and is conveniently summarized (as far as North American birds are concerned) in such works as the American Ornithologists' Union's "Check-List of North American Birds," Ridgway and Friedmann's "Birds of North and Middle America," Bent's "Life Histories of North American Birds," and various other readily available handbooks, "State Lists," etc.

How easy it is for the explorer of avian physiology, genetics or behavior to ignore the implications of this body of knowledge! For some time, we of the "Great American Desert" have smiled quietly at statements that English or House Sparrows (*Passer domesticus* [Linn.]) die rapidly at temperatures above 40° C. (104° F.). It is doubtless our fault that these have been repeated again

and again, not only by their author but more recently by another (Calhoun, 1947), since none of us has published a rebuttal.

What are the facts in this case? English Sparrows are now permanent residents at all cities and many ranches and villages throughout the arid Southwest, down to the very shores of the Gulf of California and the floor of Death Valley, and south far into Mexico. There are ample records to document the widespread popular knowledge that summer temperatures in the lower parts of the Southwest are high. Temperatures may vary several degrees in a very short distance without change of altitude (Smith, 1930, 1945; Vorhies, 1945); but shade temperatures well over 40° C. are common, in fact usual, on summer days below 500 meters of elevation. Temperatures over 50° C. have been recorded in various parts of western Arizona. Eastern California is even hotter, and I have seen a thermometer standing at 50° C. at about 6:00 in the evening at Barstow in mid-August. Yet no part of the Southwest appears to be devoid of English Sparrows because of its heat. Nor do these immigrants seem to suffer from the summer heat any more than do many native birds of the region.

While the absolute maxima may be of limited duration, temperatures only slightly lower may last for several hours. The following are official Fahrenheit temperatures at the University of Arizona, in Tucson (elevation, 2423 feet) at different times on July 1, 1947, a very hot day): 9 a.m., 92; 10 a.m., 100; 11 a.m., 104; 12 m., 107; 1 p.m., 106.2; 2 p.m., 111.1; 3 p.m., 111; 4 p.m., 108.1; 5 p.m., 108.4. At dark a hot wind came up, and the temperature during the night dropped only to 87°, rising to 108° on July 2. The same morning (July 2), the official minimum at Davis-Monthan airport, east of and higher than town, was 87.5°, while the lowest reading I could obtain with a small portable thermometer at sunrise was 85°

in a patch of grass. This was at my home adjacent to the university. Thus, despite its altitude of 750 meters, the Tucson area generally remained over 30° C. for more than 30 hours, and for about seven of these the temperature remained over 40° C. continuously, with a maximum of 44°. Yet no dead English Sparrows were found by, or reported to, me. In hot weather, birds generally (including English Sparrows) sit in the shade with their beaks open.

I have never found a free adult bird killed by heat in Arizona, though Mr. Lyndon L. Hargrave has found that, even at about 2250 meters altitude near Flagstaff, they may succumb in some 30 minutes when caged in the sun. On the other hand, a sudden summer shower with accompanying wind may drench the plumage and *chill* a bird to death. Many House Finches (*Carpodacus mexicanus frontalis* [Say]) were found dead or helpless after a shower at Tucson, June 28, 1938, by Dean Amadon and the writer. The only rumor that has reached my ears of wild birds' dying from heat pertains to Cedar Waxwings (*Bombycilla cedrorum* Vieillot); these birds are known to become poisoned (drunk) at times from eating decayed berries, so their death may have been at least partly due to this cause. It should also be noted that few birds retire to such cool microclimates as do most rodents (Vorhies, *op cit.*).

It is therefore clear that other factors must have entered into the experimental results obtained by Kendeigh (1934). Furthermore, none of the factors listed by him on page 408 as controlling migration account for those birds that winter near the Equator, nor for those that start migrating by mid-July; various passerine birds and many shore-birds migrate at this time, while some hummingbirds start south by the last of June. Recent research has shown that the Montezuma Horned Lark (*Eremophila alpestris occidentalis* [McCall]) arrives in flocks on the breeding grounds of another race by mid-June (van Rossem, 1947), and that the Anna Humming-

bird (*Calypte anna* [Lesson]) leaves its "winter" range principally in December (Phillips, 1947).

Theorizing, combined with careful testing and research, is important in the advance of science; but let us not repeat endlessly all-inclusive theories that do not fit the known facts of nature's vast outdoor laboratory.

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